CASE DB17/Div1

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF

WITTEKIND ET AL.

APPLICATION NO: UNKNOWN

FILED: HEREWITH

FOR: MODIFIED FORMS OF HEPATITIS C NS3 PROTEASE FOR FACILITATING INHIBITOR SCREENING AND STRUCTURAL STUDIES OF PROTEASE-INHIBITOR COMPLEXES

Art Unit:

Examiner:

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

This preliminary amendment is filed in conjunction with a Divisional Application of U.S. Serial No. 09/478,479 filed January 6, 2000.

In the Specification:

Please replace the paragraph at page 1, lines 4-6 with the following re-written paragraph:

-- This application is a Divisional of 09/478,479 filed January 6, 2000.--

Please replace the paragraph that begins on page 2, line 3, with the following rewritten paragraph:

-- Hepatitis C virus is a positive-strand RNA virus of the family *Flaviviridae*. The HCV genome encodes a single polyprotein of 3033 amino acids, of which residues 1027 to 1657 (631 amino acids) represent the NS3 protein (Choo et al., 1991). The HCV NS3 protein is a site-specific protease that cleaves the HCV polyprotein selectively at four sites related by their primary amino acid sequences (Grakoui et al., 1993a). These cleavages give rise to the mature non-structural (replicative) proteins of HCV, including NS3, NS4A, NS4B, NS5A, and NS5B (Bartenschlager et al., 1993; Grakoui et al., 1993b; Hijikata, et al., 1993a,b; Tomei et al., 1993; Bartenschlager et al., 1994; Eckart et al., 1994; Lin et al., 1994; Manabe, et al 1994). Genetic studies have demonstrated that the homologous NS3

proteases of related viruses (e.g. Yellow Fever Virus and Bovine viral diarrhea virus) are absolutely essential for viral replication (Chambers et al., 1990; Xu et al., 1997). Thus, inhibitors of NS3 protease should inhibit HCV replication and would be useful for the discovery and development of effective antiviral treatments for HCV infection.--

Please replace the paragraph that begins on page 9, line 1 with the following rewritten paragraph:

-- "HCV NS3" refers to the protein fragment of the HCV polyprotein from any wild type strain of HCV that corresponds to residues 1027 - 1657 of the HCV polyprotein (as defined in Choo et al. *Proceedings of the National Academy of Sciences USA* **88**, 2451-2455 [1991]). The numbering convention for HCV NS3 throughout this application starts with residue 1 corresponding to residue 1027 of the HCV polyprotein, which is the first amino-acid residue of the mature processed NS3 protein fragment. HCV NS3 has portions which confer protease activity, helicase activity, and ATPase activity. --

Please replace the paragraph that begins on page 22 at line 2 with the following rewritten paragraph:

-- The HCV NS3-encoding DNA used as a basis for all the subsequent modifications is a synthetic gene coding for the HCV protease (residues 1-181) shown in SEQ ID NO: 2 (Figure 9). Residues 1-181 per Choo et al. correspond to Residues 2-182 in SEQ ID NO:1 and in Figure 9. Residues 1-181 comprise the portion of the HCV NS3 gene product that exhibits protease activity. Longer fragments of the HCV NS3 protein could be used. The synthetic gene was constructed so that all codons were optimized for high level expression in *E.coli*. The protein-coding sequence of this construct is shown in SEQ ID NO:1 (Figure 9). This HCV protease protein is produced at a high level when expressed from vector pET24a (Novagen) in *E.coli* strain BL21(DE3) (Novagen), but upon fractionation of the extract the protease is in the insoluble fraction (data not shown).--

In the Claims:

Please cancel claims 1-23, 29, and 36-37.

Please amend claims 24, 25, 26, 30, 34 and 35 as follows:

24. (amended). A nucleic acid molecule comprising a nucleotide sequence coding for a modified HCV NS3 protease comprising at least one substitution in HCV NS3 protease of a hydrophobic α-helix 0 amino acid residue to a hydrophilic amino acid residue, wherein said modified HCV NS3 protease exhibits protease activity, or the complement thereof.

25. (amended). A nucleic acid molecule comprising a nucleotide sequence coding for a modified HCV NS4a-NS3 protease comprising the modified HCV NS3 protease of claim 24 fused to a HCV NS4a or modified HCV NS4a, wherein said modified HCV NS4a comprises residues 21-31 of full length HCV NS4a as shown in SEQ ID NO:26 having NS4a residue 30 substituted to Asn, or the complement thereof.

26. (amended). A nucleic acid molecule of claim 25 wherein the nucleotide sequence is selected from the group consisting of: SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, and SEQ ID NO:23, or the complement thereof.

30. (amended). A vector comprising a nucleic acid molecule of claim 24, 25 or 26.

34. (amended). A method for producing a modified NS3 protease comprising:

a) culturing a host cell comprising a vector comprising a nucleic acid of claim 24 under suitable conditions so as to produce the modified NS3 protease; and

b) recovering the modified NS3 protease so produced.

35. (amended). A method for producing a modified NS4a-NS3 protease comprising:

a) culturing a host cell comprising a vector comprising a nucleic acid of claim 25 under suitable conditions so as to produce the modified NS4a-NS3 protease; and

b) recovering the modified NS4a-NS3 protease so produced.

Please add claims 38-41 as follows:

38. A nucleic acid molecule of claim 24, wherein said at least one substitution is of a hydrophobic α-helix 0 amino acid residue selected from the group consisting of Leu_{13}, Leu_{14}, Ile_{17}, Ile_{18}, and Leu_{21}, wherein Leu_{13}, Leu_{14}, Ile_{17}, Ile_{18}, and Leu_{21} correspond respectively to residues 14, 15, 18, 19, and 22 of SEQ ID NO:1, or the complement thereof.

39. A nucleic acid molecule of claim 24, wherein said HCV NS3 protease comprises residues 2-182 of the amino acid sequence shown in SEQ ID NO:1 or comprises a portion of wild type HCV NS3 that confers protease activity and that differs from residues 2-182 of the amino acid sequence as shown in SEQ ID NO:1 by the inclusion or deletion of residues at either the N- or C- terminus, or the complement thereof.

40. A nucleic acid molecule of claim 25, wherein said modified HCV NS4a-NS3 fusion protease comprises a modified HCV NS3 protease of claim 39 fused to a HCV NS4a or a modified HCV NS4a, wherein said modified HCV NS4a comprises residues 21-31 of full length HCV NS4a as shown in SEQ ID NO:26 having NS4a residue 30 substituted to Asn, or the complement thereof.

41. A nucleic acid molecule of claim 25 wherein said modified HCV NS4a-NS3 fusion protease comprises an amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, and SEQ ID NO:22, or the complement thereof.

Remarks

After entry of these amendments, claims 24-28, 30-35, and 38-41 are pending in this application.

Remarks concerning amendments to the specification:

The specification has been amended on page 1 to recite that this application is a divisional of 09/478,479 fled January 6, 2000.

The specification has been amended on page 2, line 5 and on page 9, lines 2 and 5 to correct a typographical error regarding the residues in Choo et al., 1991 representing the NS3 protein. Applicants had erroneously stated that the residues representing the NS3 protein were 1026 to 1657, rather than 1027 to 1657. Applicants assert that this was an obvious error recognizable by one of ordinary skill in the art, and that the amendment adds no new matter.

That it is an obvious error is clear from the following. Prior to this amendment, the specification stated at page 9, lines 1-8, that:

> "HCV NS3" refers to the protein fragment of the HCV polyprotein from any wild type strain of HCV that corresponds to residues 1026 - 1657 of the HCV polyprotein (as defined in Choo et al. *Proceedings of the National Academy of Sciences USA* **88**, 2451-2455 [1991]). The numbering convention for HCV N53 throughout this application starts with residue 1 corresponding to residue 1026 of the HCV polyprotein, which is the first amino-acid residue of the mature processed NS3 protein fragment. HCV NS3 has portions which confer protease activity, helicase activity, and ATPase activity.

References to specific amino acids at specific locations are made at various places in the specification. For instance, at page 12, lines 7-8, Leu13 and Leu21 are referred to. At page 13, lines 16-22, Leu13, Leu14, Ile17, Ile18, and Leu21 are referred to. It is obvious to one of ordinary skill in the art looking at the sequence in Choo et al. that if Leu is

at 13, Leu is at 14, Ile is at 17, Ile is at 18, and Leu is at 21, then residue 1 is the Ala that is at 1027 in Choo et al. Thus Applicants assert that the amendment of "1026" to "1027" merely corrects an obvious error.

This specification has been amended on page 22, in the paragraph that begins at line 2 to add the following sentence: "Residues 1-181 per Choo et al. correspond to residues 2-182 in SEQ ID NO: 1 and in Figure 9." This sentence has been added for clarification, and adds no new matter. One of ordinary skill in the art can clearly see that residues 1-181, which correspond to 1027 to 1657 of Choo, correspond to residues 2-182 of SEQ ID NO: 1. Residue 1 of SEQ ID NO: 1 is a methionine that was added in order to express the peptide that follows it.

The specification has been amended on page 9, line 4, to correct a typographical error in which NS3 was mistakenly written as N53.

Remarks concerning amendments to the claims:

Claims 1-23, 29, and 36-37 have been cancelled. Claims 24, 25, 26, 30, 34 and 35 have been amended. Claims 38-41 have been added.

Support for these amendments can be found throughout the specification, including the examples. No new matter is added.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version With Markings To Show Changes Made**".

If the Examiner wishes to discuss any aspect of this case, they are invited to contact the undersigned attorney at the telephone number below.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 19-3880 in the name of Bristol-Myers Squibb Company.

Respectfully submitted,

Audrey F. Sher

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Date: September 26, 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

Paragraph beginning at line 4 on page1 has been amended as follows:

-- This application [claims priority from provisional U.S. Application Serial No. 60/115,271 filed January 8, 1999, which is incorporated herein by reference in its entirety] is a Divisional of 09/478,479 filed January 6, 2000.--

Paragraph beginning at page 2, line 3, has been amended as follows:

-- Hepatitis C virus is a positive-strand RNA virus of the family *Flaviviridae*. The HCV genome encodes a single polyprotein of 3033 amino acids, of which residues [1026]1027 to 1657 (631 amino acids) represent the NS3 protein (Choo et al., 1991). The HCV NS3 protein is a site-specific protease that cleaves the HCV polyprotein selectively at four sites related by their primary amino acid sequences (Grakoui et al., 1993a). These cleavages give rise to the mature non-structural (replicative) proteins of HCV, including NS3, NS4A, NS4B, NS5A, and NS5B (Bartenschlager et al., 1993; Grakoui et al., 1993b; Hijikata, et al., 1993a,b; Tomei et al., 1993; Bartenschlager et al., 1994; Eckart et al., 1994; Lin et al., 1994; Manabe, et al 1994). Genetic studies have demonstrated that the homologous NS3 proteases of related viruses (e.g. Yellow Fever Virus and Bovine viral diarrhea virus) are absolutely essential for viral replication (Chambers et al., 1990; Xu et al., 1997). Thus, inhibitors of NS3 protease should inhibit HCV replication and would be useful for the discovery and development of effective antiviral treatments for HCV infection.--

Paragraph beginning on page 9, line 1, has been amended as follows:

-- "HCV NS3" refers to the protein fragment of the HCV polyprotein from any wild type strain of HCV that corresponds to residues [1026] 1027 - 1657 of the HCV polyprotein (as defined in Choo et al. *Proceedings of the National Academy of Sciences USA* **88**, 2451-2455 [1991]). The numbering convention for HCV [N53] NS3 throughout this application starts with residue 1 corresponding to residue [1026] 1027 of the HCV polyprotein, which is the first amino-acid residue of the mature processed NS3 protein fragment. HCV NS3 has portions which confer protease activity, helicase activity, and ATPase activity. --

Paragraph beginning on page 22, line 2, has been amended as follows:

-- The HCV NS3-encoding DNA used as a basis for all the subsequent modifications is a synthetic gene coding for the HCV protease (residues 1-181) shown in SEQ ID NO: 2 (Figure 9). Residues 1-181 per Choo et al. correspond to Residues 2-182 in SEQ ID NO:1 and in Figure 9. Residues 1-181 comprise the portion of the HCV NS3 gene product that

exhibits protease activity. Longer fragments of the HCV NS3 protein could be used. The synthetic gene was constructed so that all codons were optimized for high level expression in *E.coli*. The protein-coding sequence of this construct is shown in SEQ ID NO:1 (Figure 9). This HCV protease protein is produced at a high level when expressed from vector pET24a (Novagen) in *E.coli* strain BL21(DE3) (Novagen), but upon fractionation of the extract the protease is in the insoluble fraction (data not shown).--

In the Claims

Claims 24, 25, 29, 30, 34 and 35 have been amended as follows:

24. (amended). A nucleic acid molecule comprising a nucleotide sequence coding for a modified HCV NS3 protease [of claim 1] comprising at least one substitution in HCV NS3 protease of a hydrophobic α-helix 0 amino acid residue to a hydrophilic amino acid residue, wherein said modified HCV NS3 protease exhibits protease activity, or the complement thereof.

25. (amended). A nucleic acid molecule comprising a nucleotide sequence coding for a modified HCV NS4a-NS3 protease [of claim 13] comprising the modified HCV NS3 protease of claim 24 fused to a HCV NS4a or modified HCV NS4a, wherein said modified HCV NS4a comprises residues 21-31 of full length HCV NS4a as shown in SEQ ID NO:26 having NS4a residue 30 substituted to Asn, or the complement thereof.

26. (amended). A nucleic acid molecule of claim 25 wherein the nucleotide sequence is selected from the group consisting of: SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, and SEQ ID NO:23, or the complement thereof.

30. (amended). A vector comprising [the] a nucleic acid molecule of claim 24, 25[,] or -26 [or 29].

34. (amended). A method for producing a modified NS3 protease comprising:

a) culturing [the] a host cell [of claim 1] comprising a vector comprising a nucleic acid of claim 24 under suitable conditions so as to produce the modified NS3 protease; and

b) recovering the modified NS3 protease so produced.

35. (amended). A method for producing a modified NS4a-NS3 protease comprising:

a) culturing [the] a host cell [of claim 13] comprising a vector comprising a nucleic acid of claim 25 under suitable conditions so as to produce the modified NS4a-NS3 protease; and

b) recovering the modified NS4a-NS3 protease so produced.

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Assistant Commissioner for Patents
Washington, D.C. 20231

SUBMISSION OF SEQUENCE LISTING
INCLUDING STATEMENT OF VERIFICATION

Sir:

Applicants hereby provide a Computer Readable Form of the Sequence Listing as well as the Paper Copy thereof. The undersigned states that the Paper Copy and the Computer Readable Form, submitted in accordance with 37 CFR §1.821(c) and (e), respectively, are the same. This submission includes no new matter.

Respectfully submitted,

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